50. *Potential Carcinogens. Part I.* Δ^6-*Steroids.*

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Partial synthesis of some Δ^6-steroids analogous in structure to the sex hormones has been accomplished, *viz.*, 17β-acetoxyandrost-6-en-3-one and androst-6-ene-3 : 17-dione, which may be compared with testosterone acetate and androst-4-ene-3 : 17-dione respectively, and androst-6-ene-3β : 17β-diol.

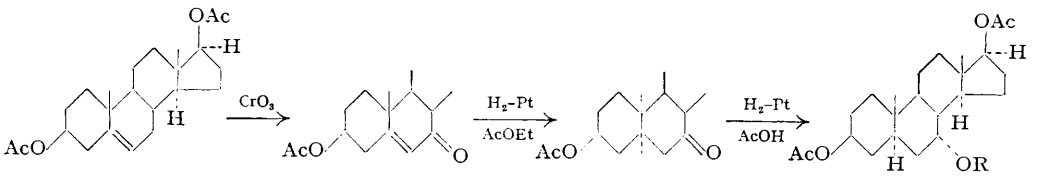
RECENT attempts to establish a connection between electron-density distribution, chemical reactivity, and carcinogenicity in unsaturated hydrocarbons (Pullman, *Compt. rend.*, 1945, **221**, 140; 1946, **222**, 392, 1501; Daudel, *ibid.*, 1946, **222**, 797; Badger, *J.*, 1949, 456; Cook and Schoental, *J.*, 1948, 170; 1950, 47) have focused attention on the phenanthrene 9 : 10-positions, termed the " K " region; the situation has been discussed by Sir Robert Robinson (*Brit. Med. J.*, 1946, 943), by Haddow (*Brit. Med. Bull.*, 1947, **4**, 331) and, more recently, reviewed by Cook (*J.*, 1950, 1210). Since the chemical reactivity shown by the 9 : 10-positions in phenanthrene is ethylenic in character (cf. Hunsberger, Ketcham, and Gutowsky, *J. Amer. Chem. Soc.*, 1952, **74**, 4839), it seemed of interest to examine the growth-inhibitory properties of steroids containing an ethylenic linkage at the Δ^6-position. There appears to be a qualitative relation between growth inhibition and carcinogenic activity (Haddow, Harris, and Kon, *Biochem. J.*, 1945, **39**, ii), and the steroids selected were analogous to the sex hormones some of which are known to be implicated in some way in the changes leading to neoplasia.

An isolated Δ^6-linkage was first introduced into the steroid nucleus by pyrolysis of 3-acetoxycholestan-7α-yl benzoate (Barton and Rosenfelder, *J.*, 1949, 2459; Wintersteiner and Moore, *J. Amer. Chem. Soc.*, 1950, **72**, 1923; cf. Plattner, Heusser, Troxler, and Segré, *Helv. Chim. Acta*, 1948, **31**, 852); since homogeneous, mechanistically unimolecular, thermal elimination reactions require *cis*-geometry of the four centres involved [6α-H (equatorial)/7α-OBz (polar)] (Barton, *J.*, 1949, 2174; cf. Cristol and Hause, *J. Amer. Chem. Soc.*, 1952, **74**, 2193, and previous papers), the pyrolysis product was 3β-acetoxycholest-6-ene. The same compound was obtained by Barton and Rosenfelder (*loc. cit.*) by pyrolysis of 3β-acetoxycholestan-6β-yl benzoate [6β-OBz (polar)/7β-H (equatorial)]. Although the stereochemistry of the cholestane ring system forbids fulfilment of the requirement that the four centres must be coplanar to minimise the activation energy, with a probable adverse effect on yields, analogous routes to appropriate androst-6-enes were examined.

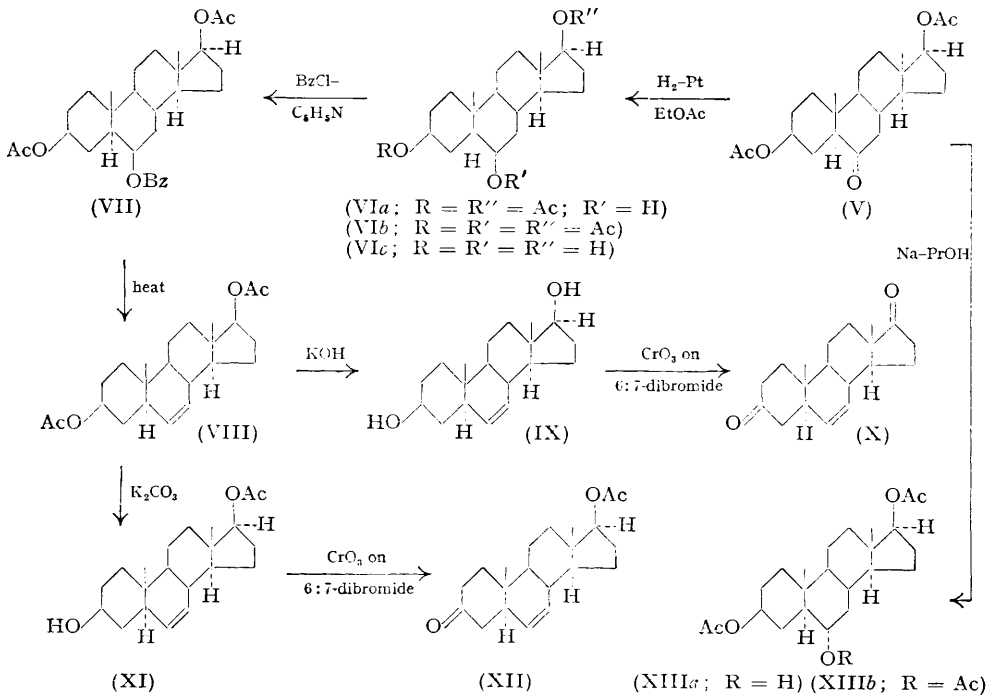
3β : 17β-Diacetoxyandrost-5-ene (I), prepared by reduction of dehydro*epi*androsterone with sodium–*n*-propanol and subsequent acetylation (Ruzicka and Wettstein, *Helv. Chim. Acta*, 1935, **18**, 1264), was oxidised by chromium trioxide to 3β : 17β-diacetoxyandrost-5-en-7-one (II) (Butenandt, Hausmann, and Paland, *Ber.*, 1938, **71**, 1316) which was hydrogenated

with platinum in ethyl acetate to 3β : 17β-diacetoxyandrostan-7-one (III), further hydrogenated with some difficulty on platinum in acetic acid to 3β : 17β-diacetoxyandrostan-7α-ol (IV*a*). The configuration at $C_{(7)}$ is supported by the molecular rotation difference $\Delta O = -74°$ since Barton and Klyne (*Chem. and Ind.*, 1948, 755) give $\Delta O\text{-}7\alpha = -59°$, $\Delta O\text{-}7\beta = +110°$; nevertheless a crystalline benzoate (IV*b*) could not be obtained and pyrolysis of the amorphous benzoate at 300—310°/0·05 mm. gave no crystalline product. The compounds (III) and (IV) are mentioned by Butenandt and Logemann in U.S.P. 2,170,124, but not described in detail.*

The alternative route to the androst-6-enes *via* the 6-substituted androstanes was more successful. 3β : 17β-Diacetoxyandrost-5-ene (I) was treated with sodium nitrite in nitric and acetic acids, to give the crude 6-nitro-derivative, which was reduced with zinc–acetic



acid to 3β : 17β-diacetoxyandrostan-6-one (V). The reduction of (V), which had to be carried out under conditions leading to a 6β-hydroxyl group without hydrolysis of the two acetoxyl groups, was accomplished by hydrogenation with platinum in ethyl acetate and



yielded 3β : 17β-diacetoxyandrostan-6β-ol (VI*a*) [cf. reduction of cholestan-6-one to cholestan-6β-ol (Shoppee and Summers, *J.*, 1952, 3361) and of 6-ketocholestan-3β-yl acetate to 6β-hydroxycholestan-3β-yl acetate (Barton and Rosenfelder, *loc. cit.*)]. Acetylation furnished 3β : 6β : 17β-triacetoxyandrostane (VI*b*), which by alkaline hydrolysis yielded

* Since this work was completed these compounds have been described by Heusler and Wettstein (*Helv. Chim. Acta*, 1952, **35**, 284).

androstane-3β : 6β : 17β-triol (VI*c*). Reduction of (V) with sodium and *n*-propanol produced, in accordance with expectation, the more thermodynamically stable 6-epimeride of (VI*c*; 6β-OH, polar), namely, androstane-3β : 6α : 17β-triol (XIII*a*; 6α-OH, equatorial), which by acetylation gave the triacetate (XIII*b*).

The configurations assigned are consistent with the molecular rotation differences found for these triols and their triacetates. Use, as reference compounds, of androstane-3β : 17β-diol, $[M]_D$ +23°, and 3β : 17β-diacetoxyandrostane, $[M]_D$ −8°, gives the molecular-rotation contributions of the 6-hydroxy- and 6-acetoxy-groups as follows :

	ΔO		ΔAc
Androstane-3β : 6β : 17β-triol	−48°	Triacetate	−105°
Androstane-3β : 6α : 17β-triol	+35°	Triacetate	+125°

Barton and Klyne (*loc. cit.*) give the following generalised values : ΔO-6β = −50°, ΔO-6α = +55°; ΔAc-6β = −110°, ΔAc-6α = +210°.

Benzoylation of (VI*a*) yielded 3β : 17β-diacetoxyandrostan-6β-yl benzoate (VII), which by pyrolysis at 250—320° gave a 20% yield of 3β : 17β-diacetoxyandrost-6-ene (VIII). Introduction of the 6-double bond produced a molecular-rotation difference of −426°; in the case of 3β-acetoxycholest-6-ene a difference of −441° was reported (Barton and Rosenfelder, *loc. cit.*). Alkaline hydrolysis of (VIII) gave androst-6-ene-3β : 17β-diol (IX), which was converted by successive bromination, oxidation with chromium trioxide–acetic acid, and debromination to androst-6-ene-3 : 17-dione (X).

Partial hydrolysis of (VIII) with potassium carbonate gave 17β-acetoxyandrost-6-en-3β-ol (XI), which was converted into the Δ^6-analogue of testosterone acetate, namely, 17β-acetoxyandrost-6-en-3-one (XII) by oxidation of the dibromide with chromium trioxide–acetic acid and subsequent debromination.

Androst-6-ene-3β : 17β-diol (IX) and -3 : 17-dione (X), and 17β-acetoxyandrost-6-en-3-one (XII), together with the related compounds Δ^6-testosterone, androsta-4 : 6-diene-3 : 17-dione, and androsta-1 : 4 : 6-triene-3 : 17-dione, which may be expected to possess androgenic activity, the œstrogen 1-methyl-19-nor-Δ^6-œstrone, and the gestagen Δ^6-progesterone, have been sent to Professor A. Haddow, The Royal Cancer Hospital, for biological testing on the rate of growth of the Walker rat carcinoma. Δ^6-Progesterone exhibited a weakly positive effect in this test; further results will be published by Professor Haddow.

Experimental

For general experimental directions see preceding paper. Microanalyses are by Drs. Weiler and Strauss, Oxford.

3β : 17β-*Diacetoxyandrostan*-7-*one* (III).—3β : 17β-Diacetoxyandrost-5-en-7-one (II) (1·2 g.; m. p. 220°), prepared from 3β : 17β-diacetoxyandrost-5-ene by the method of Butenandt *et al.* (*Ber.*, 1938, **71**, 1316), was hydrogenated in ethyl acetate (40 c.c.) with platinum oxide (30 mg.); 1 mol. of hydrogen was absorbed in 2 hr., whereafter uptake ceased. Filtration from the catalyst, removal of solvent, and crystallisation of the residue from ethanol yielded 3β : 17β-*diacetoxyandrostan*-7-*one* (0·90 g.), m. p. 195—197°, $[\alpha]_D$ −41° ± 2° (*c* = 1·19)* (Found, after drying at 20°/0·01 mm. : C, 70·5; H, 8·8. $C_{23}H_{34}O_5$ requires C, 70·7; H, 8·8%). An additional quantity (0·21 g.), m. p. 190—192°, was obtained from the mother-liquors. The *semicarbazone* had m. p. 274—276° (Found, after drying at 100°/0·01 mm. for 1 hr. : N, 8·9. $C_{24}H_{37}O_5N_3$ requires N, 9·4%).

3β : 17β-*Diacetoxyandrostan*-7α-*ol* (IV*a*).—3β : 17β-Diacetoxyandrostan-7-one (1 g.) was hydrogenated with platinum oxide (60 mg.) in acetic acid (20 c.c.); 1 mol. of hydrogen was absorbed in 3 hr. After removal of the catalyst, acetic acid was evaporated off in a vacuum, and the residue dissolved in ether, washed with 2N-sodium carbonate and water, and dried. After evaporation, crystallisation from acetone–hexane yielded 3β : 17β-*diacetoxyandrostan*-7α-*ol*, m. p. 188—190°, $[\alpha]_D$ −21° ± 2° (*c* = 2·37)* (Found, after drying at 60°/0·01 mm. for 18 hr. : C, 70·15; H, 9·0. $C_{23}H_{36}O_5$ requires C, 70·35; H, 9·25%). The alcohol was benzoylated in pyridine at 15° for 24 hr. but the product could not be crystallised.

3β : 17β-*Diacetoxyandrostan*-6-*one* (V).—3β : 17β-Diacetoxyandrost-5-ene (I) (m. p. 159—

* Heusler and Wettstein (*Helv. Chim. Acta*, 1952, **35**, 284) have since recorded m. p. 193°, $[\alpha]_D$ −39° ± 4°, and m. p. 186°, $[\alpha]_D$ − 15° ± 4° respectively.

161°; 5·3 g.) was dissolved in acetic acid (75 c.c.), and fuming nitric acid (30 c.c.) was added in portions with cooling. Sodium nitrite (3·0 g.) was added in small portions during 1 hour at < 22°. The mixture was poured into water; the precipitated 3β : 17β-diacetoxy-6-nitro-androst-5-ene, when washed with water and dried in a vacuum-desiccator, had m. p. 85—95°, but could not be recrystallised. The crude 6-nitro-compound was dissolved in acetic acid (24 c.c.) containing water (5 c.c.), and zinc dust (10 g.) was added in portions; the solution became warm and was refluxed for 4 hr., cooled, and filtered, the filtrate partially evaporated in a vacuum, and the product poured into water. The crystalline ketone was dried and recrystallised from methanol, to yield material (2·9 g.), m.p. 164—176°. Repeated recrystallisation from methanol gave 3β : 17β-*diacetoxyandrostan*-6-*one*, m. p. 176—178°, $[\alpha]_D$ −37° ± 2° (*c* = 3·58) (Found, after drying at 20°/0·05 mm. for 16 hr.: C, 70·5; H, 8·75. $C_{23}H_{34}O_5$ requires C, 70·7; H, 8·8%). The mother-liquors from several reductions were combined and chromatographed on aluminium oxide with elution by benzene and benzene–ether (4 : 1) to give additional material.

3β : 17β-*Diacetoxyandrostan*-6β-*ol* (VI*a*).—3β : 17β-Diacetoxyandrostan-6-one (6·7 g.) in ethyl acetate (150 c.c.) was shaken with platinum oxide (600 mg.) in hydrogen; 0·78 mol. of hydrogen was absorbed in 90 min. whereafter absorption became very slow. After filtration from the catalyst and evaporation in a vacuum, the residue was chromatographed on aluminium oxide (180 g.) in benzene–pentane (1 : 2) with 600-c.c. eluant batches. Pentane–benzene (1 : 1) (2 fractions) and then benzene (fraction 3) removed mainly the starting ketone. Further elution with benzene (fractions 4—6) gave material, m. p. 110—125° (1·23 g.), and elution with ether–benzene (1 : 4) (fractions 7—9) gave material of m. p. 100—125° (3·25 g.). Fractions 4—9 were united and recrystallised from hexane, to yield 3β : 17β-*diacetoxyandrostan*-6β-*ol*, m. p. 128—130°, $[\alpha]_D$ −30° ± 2° (*c* = 2·17) (Found, after drying at 20°/0·01 mm. for 16 hr.: C, 70·45; H, 9·25. $C_{23}H_{36}O_6$ requires C, 70·35; H, 9·25%). Oxidation of the alcohol with chromium trioxide yielded the ketone (V), m. p. 176—178°, mixed m. p. 176—178°. Acetylation with pyridine–acetic anhydride at 20° gave 3β : 6β : 17β-*triacetoxyandrostane* (VI*b*), needles (from pentane), m. p. 74—75°, $[\alpha]_D$ −26° ± 4° (*c* = 0·65) (Found, after drying at 25°/0·05 mm. for 18 hr.): C, 69·0; H, 8·95. $C_{25}H_{38}O_6$ requires C, 69·05; H, 8·8%), alkaline hydrolysis of which yielded androstane-3β : 6β : 17β-triol (VI*c*), which forms a *hydrate*, m. p. 135° with immediate resolidification and final melting at 222—224° after crystallisation from benzene, $[\alpha]_D$ −8° ± 3° (*c* = 0·89). The substance could not be satisfactorily dried and a good analysis could not be obtained for the anhydrous triol (Found, after drying at 20°/0·01 mm. for 11 hr.: C, 70·0; H, 10·1. $C_{19}H_{32}O_3,H_2O$ requires C, 69·9; H, 10·5%).

Androstane-3β : 6α : 17β-*triol*.—3β : 17β-Diacetoxyandrostan-6-one (138 mg.) was dissolved in *n*-propanol (10 c.c.), and sodium (0·6 g.) added to the refluxing solution during 1 hr. The resulting solution was cooled and neutralised with 2N-hydrochloric acid, most of the propanol removed by distillation in a vacuum, and the residue diluted with water to yield androstane-3β : 6α : 17β-triol, m. p. 244—246° after crystallisation from benzene, $[\alpha]_D$ +27° ± 2° (*c* = 1·72). Satisfactory analytical results could not be obtained on account of its hygroscopic nature (Found, after sublimation at 200°/0·01 mm.: C, 67·2; H, 9·7%). Acetic anhydride–pyridine at 20° yielded 3β : 6α : 17β-*triacetoxyandrostane*, m. p. 64—68° after crystallisation from aqueous methanol, $[\alpha]_D$ +19° ± 2° (*c* = 1·77) (Found, after drying at 25°/0·05 mm. for 18 hr.: C, 69·15; H, 8·85. $C_{25}H_{38}O_6$ requires C, 69·05; H, 8·8%).

3β : 17β-*Dicetoxyandrostan*-6β-*yl Benzoate* (VII).—3β : 17β-Diacetoxyandrostan-6β-ol (VI*a*) (5·3 g.) was dissolved in dry pyridine (20 c.c.), benzoyl chloride (5 c.c.) added, and the mixture set aside at 20° for 24 hr. The solution was poured into water and next morning was extracted with ether; the ethereal solution was washed with 2N-hydrochloric acid, 2N-sodium carbonate, and water, dried, and evaporated. The amorphous residue did not readily crystallise, but after filtration in benzene through aluminium oxide the product crystallised from ether, to yield crude 3β : 17β-*diacetoxyandrostan*-6β-*yl benzoate*, m. p. 94—100°; recrystallisation from ether raised the m. p. to 101°, $[\alpha]_D$ −67° ± 2° (*c* = 3·84) (Found, after drying at 20°/0·05 mm. for 18 hr.: C, 72·7; H, 8·05. $C_{30}H_{40}O_6$ requires C, 72·55; H, 8·1%).

3β : 17β-*Diacetoxyandrost*-6-*ene* (VIII).—3β : 17β-Diacetoxyandrostan-6β-yl benzoate (5·3 g.) was heated in a distillation flask in a stream of carbon dioxide at 16 mm.; when the bath-temperature reached 310°, the bath was removed, the flask heated with a naked flame, and the contents distilled rapidly at 0·05 mm. The distillate, partly crystalline, was dissolved in ether, and the ethereal solution washed with 2N-sodium carbonate and water, dried, and evaporated. The residue (4 g.) crystallised from benzene–pentane (1 : 4), yielding 3β : 17β-diacetoxyandrost-6-ene (615 mg.), m. p. 174—178°. The mother-liquor was evaporated and the amorphous residue (3·4 g.) chromatographed on aluminium oxide (120 g.) prepared in benzene–pentane (1 : 4) with

eluant batches of 350 c.c. Elution with benzene–pentane (1 : 4 and 2 : 5) yielded uncrystallisable oils probably containing androsta-2 : 6-dien-17β-yl acetate, androsta-6 : 16-dien-3β-yl acetate, and possibly androsta-2 : 6 : 16-triene. The first eluate obtained with benzene–pentane (1 : 1) was oily, but the subsequent 5 fractions obtained with this mixture crystallised (m. p. 165—173°); these fractions were united and recrystallised from acetone, to give 3β : 17β-*diacetoxyandrost*-6-*ene*, m. p. 176—180° (total yield 20%). For analysis a specimen, recrystallised from ethanol, m. p. 178—180°, $[\alpha]_D$ −116° ± 2° (*c* = 4·56), giving a yellow colour with tetranitromethane–chloroform, was used (Found, after drying at 20°/0·05 for 16 hr.: C, 74·0; H, 9·2. $C_{23}H_{34}O_4$ requires C, 73·75; H, 9·15%).

Hydrolysis of the diacetate with potassium hydroxide in methanol yielded androst-6-ene-3β : 17β-diol (IX), m. p. 181—182° after crystallisation from hexane, $[\alpha]_D$ = −121° ± 2° (*c* = 1·02). Satisfactory analytical results could not be obtained for this substance which appeared to be hygroscopic (Found, after sublimation at 160°/0·01 mm.: C, 77·1, 76·4, 76·1; H, 10·3, 10·1, 10·8. Calc. for $C_{19}H_{30}O_2$: C, 78·6; H, 10·4%).

Androst-6-*ene*-3 : 17-*dione* (X).—To androst-6-ene-3β : 17β-diol (477 mg.) in acetic acid (40 c.c.), bromine in acetic acid was added dropwise until the colour was no longer discharged. A 4% solution of chromium trioxide in 95% acetic acid (9 c.c.) was then added and the mixture kept at 20° for 18 hr. Excess of chromic acid was destroyed with methanol, and the acetic acid largely removed at 30° in a vacuum. The precipitate obtained on addition of a large volume of water was filtered off, washed with water, and dried in a vacuum-desiccator; it was then debrominated with zinc dust in acetic acid at 100° for 15 min. After removal of zinc the filtrate was evaporated to small volume in a vacuum and diluted with water. The crystalline product was dissolved in benzene and filtered through aluminium oxide; crystallisation from ethanol gave *androst*-6-*ene*-3 : 17-*dione* (362 mg.), m. p. 165—167°, $[\alpha]_D$ −31° ± 2° (*c* = 1·78) (Found, after drying at 20°/0·05 for 18 hr.: C, 79·5; H, 9·3. $C_{19}H_{26}O_2$ requires C, 79·7; H, 9·15%). The diketone was characterised by the *dioxime*, m. p. 257—258° after crystallisation from ethyl acetate (Found, after drying at 20°/0·05 mm. for 16 hr.: N, 8·3. $C_{19}H_{28}O_2N_2$ requires N, 8·85%).

17β-*Acetoxyandrost*-6-*en*-3β-*ol* (XI).—3β : 17β-Diacetoxyandrost-6-ene (V) (363 mg., 0·97 mmole) in methanol (30 c.c.) was treated with potassium carbonate (70 mg., 0·51 mmole) in water (2·0 c.c.) and methanol (4·0 c.c.), and the solution set aside at 15° for 18 hr. The small amount of residual potassium carbonate was neutralised with acetic acid and the solvent removed in a vacuum; the residue was dissolved in ether and the ethereal solution washed with sodium hydrogen carbonate solution and with water, dried, and evaporated. The product was chromatographed on aluminium oxide (9 g.) prepared in benzene–pentane (1 : 4); elution with benzene and with ether–benzene (1 : 5) yielded the crude monoacetate (138 mg.) which, recrystallised from hexane, gave 17β-*acetoxyandrost*-6-*en*-3β-*ol* (107 mg.), m. p. 150—152°, $[\alpha]_D$ −130° ± 2° (*c* = 1·05) (Found, after sublimation at 140°/0·05 mm.: C, 76·0; H, 9·5. $C_{21}H_{32}O_3$ requires C, 75·85; H, 9·7%).

17β-*Acetoxyandrost*-6-*en*-3-*one* (XII).—To 17β-acetoxyandrost-6-en-3β-ol (368 mg.) in acetic acid (12 c.c.), bromine in acetic acid was added until a faint yellow colour persisted; a 4% solution of chromium trioxide in 95% acetic acid (3·0 c.c.) was then added and the mixture kept at 15° for 18 hr. Excess of chromium trioxide was destroyed with methanol, and the solution evaporated at 30° in a vacuum and diluted with water; the precipitated dibromo-diketone was filtered off, washed with water, and dried in a vacuum-desiccator. Debromination was effected by zinc dust in acetic acid at 100° (15 min.); the product, dissolved in benzene, was filtered through aluminium oxide and crystallised from ethanol, to give 17β-*acetoxyandrost*-6-*en*-3-*one* (263 mg.), m. p. 153—155°, $[\alpha]_D$ −96° ± 2° (*c* = 1·24) (Found, after drying at 20°/0·05 mm. for 15 hr.: C, 76·6; H, 9·2. $C_{21}H_{30}O_3$ requires C, 76·35; H, 9·15%).

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